# Increased fetuin-A levels following treatment with a vitamin D analog

**To the Editor:** Fetuin-A is one of the several potential vascular calcification inhibitors in chronic kidney disease (CKD) patients; it seems to protect from precipitation of calcium phosphate under extra-osseous calcification stress by organizing a fetuin–mineral complex (FMC).[1]

Vitamin D has been demonstrated to promote ectopic calcifications by different mechanisms that also include fetuin-A exhaustion as a result of FMC formation. This hypothesis was supported by *in vivo* evidence in rats fed with toxic doses of vitamin D,[2] which induced ectopic calcifications and reduction of circulating fetuin-A; however, this mechanism has never been investigated in humans. In addition, Matsui *et al.*[1] recently reported a slight decrease in serum fetuin-A levels and a marked rise in FMC in uremic rats with very high levels of calcium × phosphorus (Ca × P) product.

We studied fetuin-A serum level variations in hemodialysis patients suffering from secondary hyperparathyroidism, before and after the administration of a vitamin D analog (paricalcitol).

We treated 10 consecutive hemodialysis patients with hyperparathyroidism, who had never received vitamin D treatment, with i.v. paricalcitol 5 μg thrice weekly; we obtained their sera before the first paricalcitol administration (t0), and after 1 week (t1), 4 weeks (t4), and 8 weeks (t8); serum levels of fetuin-A (measured using a human fetuin-A ELISA kit, Epitope Diagnostics, San Diego, CA), high-sensitivity C-reactive protein (hsCRP), total intact parathyroid hormone (PTH), and serum calcium and phosphorus levels were measured. The results are shown in Figure 1.

Contrary to that reported in rats, we observed, comparing t8 with t0, a progressive and statistically significant increase in serum fetuin-A levels ($P = 0.01$); there was also an increase in the Ca × P product ($P = 0.42$) and a decrease in PTH ($P = 0.07$) and hsCRP levels ($P = 0.06$), but these changes were not statistically significant. Because it is well known that inflammation, as assessed by hsCRP, may condition fetuin-A synthesis, we investigated whether changes in fetuin-A correlated with changes in hsCRP; in our patients there was no significant correlation between these two parameters ($r = -0.188$, $P = 0.60$); therefore, the increase in fetuin-A we observed after paricalcitol treatment did not seem to be accounted for by changes in the inflammatory status of our patients.

The increase in fetuin-A serum levels could be due to hepatic stimulation of fetuin-A synthesis induced by vitamin D through its action on the hepatocyte vitamin D receptor. In agreement with our data, serum levels of 1–25 OH dihydroxy-vitamin D in untreated CKD patients were found to correlate with serum fetuin-A levels.[3]

In conclusion, although vitamin D may elicit vascular calcification through an increase in the Ca × P product, in humans it is apparent that it may also activate a counter-regulatory mechanism that leads to an increased production of fetuin-A. These data lend further support to the view that vitamin D may exert pleiotropic effects on different organ systems, as reviewed by Verstuyf *et al.*[4] in a recent issue of *Kidney International*.

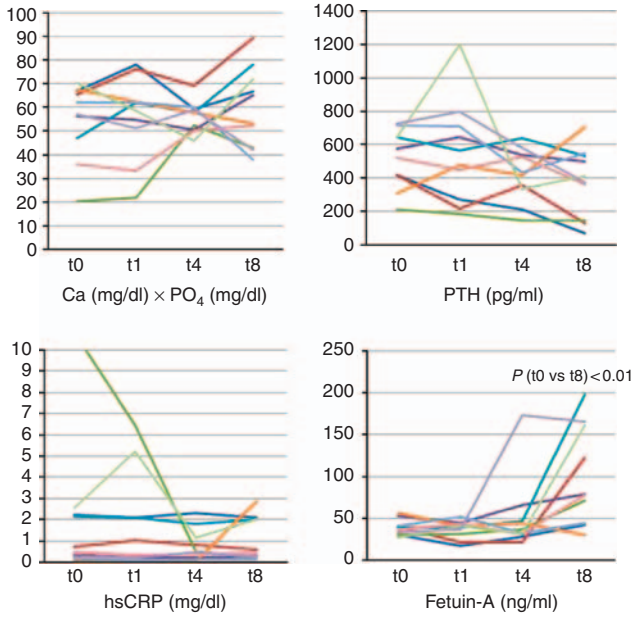


**Figure 1 | Serum levels of fetuin-A, Ca × P product, parathyroid hormone (PTH), and high-sensitivity C-reactive protein (hsCRP) in the 10 patients studied, before (t0) and after 1 week (t1), 4 weeks (t4), and 8 weeks (t8) of paricalcitol treatment.**

1. Matsui I, Hamano T, Mikami S *et al.* Fully phosphorylated fetuin-A forms a mineral complex in the serum of rats with adenine-induced renal failure. *Kidney Int* 2009; **75**: 915–928.
2. Price PA, Williamson MK, Nguyen TMT *et al.* Serum levels of the fetuin-mineral complex correlate with artery calcification in the rat. *J Biol Chem* 2004; **279**: 1594–1600.
3. Mehrotra R, Westenfeld R, Christenson P *et al.* Serum fetuin-A in nondialyzed patients with diabetic nephropathy: relationship with coronary artery calcification. *Kidney Int* 2005; **67**: 1070–1077.
4. Verstuyf A, Carmeliet G, Bouillon R *et al.* Vitamin D: a pleiotropic hormone. *Kidney Int* 2010; **78**: 140–145.

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**The Author Replies:** Manenti *et al.*[1] observed a significant increase in serum fetuin-A levels starting a few weeks after thrice-a-week paracalcitol treatment of 10 patients on chronic hemodialysis. The simple consequence could be that such

increase in fetuin-A, a major inhibitor of (extra-skeletal) calcium-phosphate deposition, may help to offset the risk of vitamin D therapy-induced increase in calcium-phosphate product. However, the observed increase as presented in their figure was much delayed, and so it is unlikely to be a direct effect of the analog treatment. Moreover, the effects of vitamin D on vascular wall and cardiovascular risks are extremely complex and involve mostly beneficial effects but also possible deleterious effects at very high vitamin D levels.[2] Ectopic vascular calcification is a complex phenomenon whereby mesenchymal cells are redirected to become osteoblast-like cells that can produce matrix and deposit minerals that then become calcified vascular lesions.[3–5] The vitamin D hormone and its analogs can indeed induce this (trans)differentiation *in vitro*, but *in vivo* results with either the parent vitamin D compound[6] or vitamin D analogs,[3] together with the beneficial effects of vitamin D analog treatment in chronic renal failure (CRF) patients[7] on overall survival and cardiovascular events, clearly demonstrate that the vitamin D effect is much more complex. A poor vitamin D status is indeed associated with increased prevalence of cardiovascular risk and events in the general population[8] as well as in CRF patients, so that correction of poor vitamin D status may improve cardiovascular risks although this requires confirmation by the appropriate randomized controlled trials (RCTs). The process of vascular mineralization is enhanced by high phosphate, oxidative stress, vitamin D, and parathyroid hormone (PTH) (fragments), and bone morphogenic proteins, but is also inhibited by inhibitors of mineralization such as fetuin-A, osteopontin, and matrix gamma-carboxyglutamate (Gla) protein. Moreover, fibroblast growth factor (FGF23) and its receptor and co-receptor Klotho are also very potent regulators of vascular function and even survival or longevity, but their precise role in the vascular function or dysfunction needs further clarification.[9,10] Therefore, the observation of Manenti *et al.* are just the very brief start of a series of much needed studies to document the process of accelerated vascular dysfunction in CRF patients so as to define the real culprit and potential natural protectors. This will allow then to better define the relative importance of control of serum calcium, phosphate, and calciotropic hormones, including FGF23 and Klotho, in the prevention of cardiovascular and mortality risks. It seems logical that only a combination of *in vitro* and *in vivo* studies in appropriate animal models and prospective RCTs in human patients will be able to provide us with the necessary answers.


1. Manenti L, Vaglio A, Pasquali S. Increased fetuin-A levels following treatment with a vitamin D analog. *Kidney Int* 2010; **78**: 1187.
2. Verstuyf A, Carmeliet G, Bouillon R, Mathieu C. Vitamin D: a pleiotropic hormone. *Kidney Int* 2010; **78**: 140–145.
3. Mizobuchi M, Towler D, Slatopolsky E. Vascular calcification: the killer of patients with chronic kidney disease. *J Am Soc Nephrol* 2009; **20**: 1453–1464.
4. Coen G, Manni M, Agnoli A *et al.* Cardiac calcifications: Fetuin-A and other risk factors in hemodialysis patients. *ASAIO J* 2006; **52**: 150–156.
5. Johnson RC, Leopold JA, Loscalzo J. Vascular calcification: pathobiological mechanisms and clinical implications. *Circ Res* 2009; **105**: e8.
6. Verberckmoes R, Bouillon R, Krempien B. Disappearance of vascular calcifications during treatment of renal osteodystrophy. Two patients treated with high doses of vitamin D and aluminum hydroxide. *Ann Intern Med* 1975; **82**: 529–533.
7. Teng M, Wolf M, Lowrie E *et al.* Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. *N Engl J Med* 2003; **349**: 446–456.
8. Wang TJ, Pencina MJ, Booth SL *et al.* Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008; **117**: 503–511.
9. Razzaque MS. The FGF23-Klotho axis: endocrine regulation of phosphate homeostasis. *Nat Rev Endocrinol* 2009; **5**: 611–619.
10. Kuro-o M. Overview of the FGF23-Klotho axis. *Pediatr Nephrol* 2010; **25**: 583–590.



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**The Authors Reply:** We thank Dr Manenti *et al.*[1] for their interest in our study. Despite no direct evidence showing the transcriptional regulation of fetuin-A by vitamin D, paricalcitol might increase fetuin-A production, given that vitamin D itself has an anti-inflammatory action and that fetuin-A is a negative acute-phase protein. The lack of significant correlation between changes in fetuin-A and those in high-sensitivity C-reactive protein cannot rule out this possibility, because the number of the patients is so small.

The effect of vitamin D on vascular calcification appears to be dependent on the dosage of vitamin D.[2] High-dose vitamin D increases calcium-phosphate product and promotes vascular calcification, whereas vitamin D at therapeutic doses can reduce calcification by suppressing aortic osteoblastic gene expression.[2] Thus, it may not be appropriate to directly compare the result from rats treated with toxic doses of vitamin D and the one from humans treated with therapeutic doses.

It is very important to take fetuin-A fractions into consideration when discussing the roles of fetuin-A. Our quoted paper[3] insisted that measuring fetuin-mineral complex (FMC) rather than fetuin-A is important for evaluating calcification stress. Recently, we found that similar FMC could be separated in the serum of patients with chronic kidney disease (CKD).[4] Our results in CKD patients indicated that serum fetuin-A levels in humans determined by enzyme-linked immunosorbent assay were the sum of the fetuin-A level bound to calcium and the free fetuin-A level, and that the former reflects ongoing calcification stress. From this perspective, further studies seem to be required to elucidate which type of fetuin-A is increased by paricalcitol.


1. Manenti L, Vaglio A, Pasquali S. Increased fetuin-A levels following treatment with a vitamin D analog. *Kidney Int* 2010; **78**: 1187.
2. Mathew S, Lund RJ, Chaudhary LR *et al.* Vitamin D receptor activators can protect against vascular calcification. *J Am Soc Nephrol* 2008; **19**: 1509–1519.